

**Osteotropism of NETs:  
Role of CXCR4 in Regulating the EMT Process**

**Francesca Maria Rizzo**<sup>1</sup>; Mauro Cives<sup>1</sup>; Valeria Simone<sup>1</sup>;  
Ilaria Maggio<sup>1</sup>; Davide Quaresmini<sup>1</sup>; Claudia Felici<sup>1</sup>; Morena  
D'Avenia<sup>1</sup>; Franco Silvestris<sup>1</sup>

<sup>1</sup>Department of Biomedical Sciences and Human Oncology,  
University of Bari, 70124 – Bari, Italy

**Background:** Neuroendocrine tumors (NETs) metastasize to the bone in up to 20% of patients with well/moderately differentiated disease. We have previously demonstrated that activation of the epithelial-to-mesenchymal transition (EMT) regulates the NET osteotropism. In particular, CXCR4 overexpression was related to increased risk of bone colonization. Here we investigated its activity in driving this process.

**Methods:** BON1, QGP1, CM, H727 and CNDT2.5 NET cell lines were evaluated by MTT test in their proliferative response to the CXCR4 ligand SDF1. The transcription and expression levels of a panel of EMT-related genes (*CXCR4*, *SNAIL*, *TGF- $\beta$ 1*, *CTGF*, *EpCAM*, *E-Cadherin*, *N-Cadherin*, *IL-11*, *RANK*) were screened by RT-PCR and immunocytochemistry at baseline and after 2hr of SDF1 treatment. Migration and invasion of NET cells towards the bone were evaluated by functional assays, while the subcellular distribution of CXCR4 was investigated by confocal and electron microscopy. Western blot (WB) was used to detect CXCR4 isoforms in nuclei. Chromatin immunoprecipitation and nuclear calcium mobilization are underway to elucidate the CXCR4 function in nuclei.

**Results:** SDF1 was inert on NET cell proliferation. EMT-related genes were constitutively up-regulated in BON1 cells ( $p < 0.01$ ), whereas their transcription and expression levels were inducible by SDF1 ( $p < 0.03$ ) in CM and H727 cell lines, but not in QGP1 and CNDT2.5 cells. Overall, there was a defined correlation between mRNA and protein levels ( $r = 0.53$ ,  $p = 0.04$ ). Only BON1 cells showed an intrinsic tropism towards the bone ( $p < 0.0001$ ), whereas CM and H727 cells significantly increased their migration only after incubation with SDF1 ( $p = 0.0025$  and  $p = 0.01$ , respectively). Following agonist stimulation, CXCR4 migrated to the nuclear membrane and nucleoli of BON1, CM and H727 cells, where its nuclear levels increased up to 240%.

**Conclusion:** Stimulation of CXCR4 dramatically enhances the osteotropism of several NET cell lines by inducing the EMT. Migration of specific CXCR4 isoforms towards the nuclei may play a role in this process.